

10.5 kb

age No. _____

to see: to try another miniprep - 10.5 kb fragment contained
in pDETA I - and amplify

used overnight cultures grown in the presence of Tetr + Kan
saved at 4°, over 6 hr old.

Alkaline lysis protocol. all resuspended in 25 µl g. To
extracted from 3 x 1.5 ml culture
Did an enzyme titration }
& Mg " } - amplification done only with
miniprep DNA, no plasmids
were used.

included was just 1.2 v with Tag 1 =

Vol 50 µl. 200 µM dNTP cycling 3 step
4, 3, 2 mM Mg (95°, 3')
{ 4 µM primer (95°, 45")
{ 2, 5 U enzyme (55°, 30") 25 cycles.
{ .01, .02, .05 } (72°, 5')

prepared primex with Buffer B containing 2 mM Mg

added supplement Mg accordingly. 2 mM 3 mM 4 mM

used 2 µl g miniprep Template
con. unchanged.

0 5 10
50 45 40

prepared 20x primex

50 50 300

x buffer 100)

1
5 µl / rx as
needed.

dNTP 20

40 x 20

enzyme 1 ml addin

P1 4

40

5 + 2 7 dilin

P2 4

5

dry. con.

40

5

enzym

(in, 5 ml)

50

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Read & Understood by me,

Date

Invented by

Date

[Signature]

1/20/95

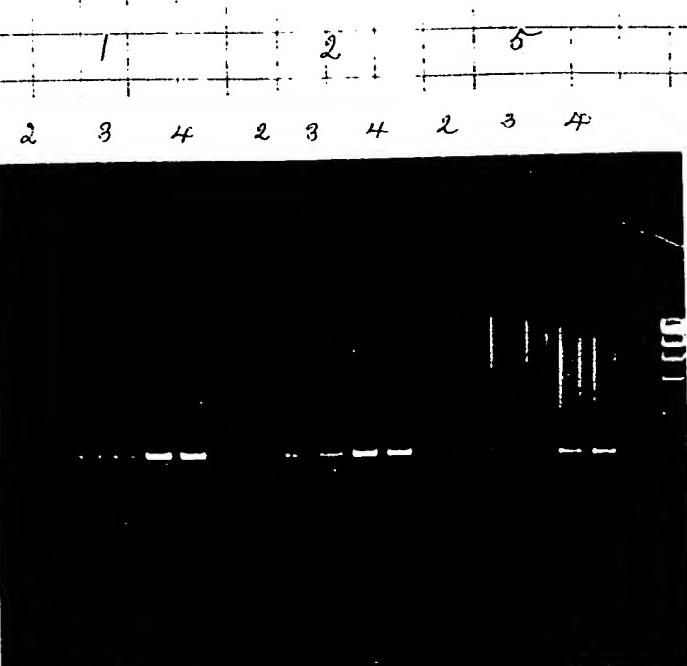
Recorded by

1/10/95

R. Subramoni

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1	2		2		
3	4	}	10	3	
5	6			4	
7	8			2	
9	10	}	20	3	
11	12			4	
13	14			2	
15	16	}	50	3	
17	18			4	
19	Tag	20	2 mrt		→



Bennell ;

cycling has to be optimized. - Job of engineering
2 m/s My didn't work in any of the sets? - It
= worked earlier in 25m as we thought.

amount of simplicity?

Increasing the enzyme didn't seem to work.
we also tryed Mg

Tag alone at 2W / 50µL didn't work

get fresh everyone.

To Page

With ~~sseed~~ & Und ~~rsto~~ d by me,

Dat

Invented by

Dat

R c rded by

L. sibarawri

1/10/1934